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Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci

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Summary. We report that plant height quantitative trait loci (QTLs) identified in a given small population are not consistent with QTLs identified in other small populations, and that most QTLs are in close proximity to mapped qualitative genetic loci. These observations provide evidence to support the hypothesis that qualitative genetic loci are the same loci that affect quantitative traits, and affirm that these modest experiments probably identify real QTLs.

Key words: *Zea mays* L. – Molecular markers – Restriction fragment length polymorphisms (RFLPs) – Quantitative trait loci (QTLs)

Introduction

Robertson (1985) suggested that loci with qualitative mutants are the same loci that affect the expression of quantitative traits, and that the qualitative mutants are mostly null or near-null alleles at quantitative trait loci (QTLs). As an example, Robertson referred to plant height as a quantitative trait with known qualitative mutants. The first qualitative mutant affecting plant height in maize (*Zea mays* L.) was identified almost 80 years ago (Emerson 1912). There have since been numerous mutants that affect plant height in maize (Sheridan 1988), and many of these have been genetically mapped (D. A. Hoisington, personal communication). Genetic characterization of these genes has been possible because phenotypic expression was a discrete variable with easily identifiable Mendelian classes. However, in most germ plasm used by plant breeders, phenotypic expression is usually a continuous variable. This continuous variability has been attributed to polygenic inheritance of several QTLs, an array of alleles possible at each QTL, and the ever-present environmental modulation of genetic affects (Robertson 1985; Jensen 1989).

With the development of molecular markers such as isozymes and RFLPs, maize QTLs for plant height have been identified (Edwards et al. 1987). T. Helentjaris and D. Shattuck-Eidens (personal communication) showed that a QTL for plant height in a wide cross, Tx303 × CO159, was identified in close proximity to a gibberellic acid (GA) dwarf locus, *d3*. The authors did not indicate whether additional QTLs also exhibited associations with other known qualitative genetic loci. Another important question is whether or not the QTL associated with *d3* in Tx303 × CO159 can be identified in other germ plasm.

We have identified plant height QTLs in four F_2 maize populations and report the information along with their proximity to mapped qualitative genetic loci. This information provides evidence, albeit circumstantial, in support of Robertson's hypothesis on the relationship of qualitative mutants to quantitative traits. The information also shows the numbers and genomic distribution of plant height QTLs in elite corn belt maize.

Materials and methods

Segregation data on 209 genetic markers were obtained from four F_2 populations (Table 1). Most of these genetic markers are restriction fragment length polymorphisms (RFLPs) revealed by *Pst*I genomic probes and were named according to the conventions proposed by E.H. Coe and D. A. Hoisington (personal communication). Six of the genetic markers used were isozyme and DNA probes from identified genes. RFLP data were obtained using DNA extracted from either F_2 plants or from six to ten pooled F_3 or F_4 plants, according to Saghai-Maroof et al. (1984). Restriction enzyme digestions, gel electrophoresis, transfer of DNA to nylon membranes, and DNA hybridizations were

Table 1. Number of progeny, genetic markers, and estimated genome size of four maize populations

Population	No. of progeny	No. of genetic markers	Estimated genome size (cM)
B73 × MO17	112	148	2,200
B73 × G35	112	106	2,100
K05 × W65	144	78	1,600
J40 × V94	144	68	1,500

Table 2. Repeatability among $F_{2:4}$ lines and average plant height for four maize populations derived from inbreds adapted to the U.S. Corn Belt

Population	Repeatability	Average plant height (cm)			
		Parent 1	Parent 2	F_1	$F_{2:4}$ lines
B73 × MO17	0.85	219	208	244	229
B73 × G35	0.90	221	201	244	221
K05 × W65	0.91	147	190	208	188
J40 × V94	0.88	145	231	226	198

accomplished using standard procedures (Sambrook et al. 1989).

Genetic linkage maps of 68–148 genetic markers (Table 1) were constructed for each population using MAPMAKER (Lander et al. 1987). QTLs were identified in each population using interval mapping and multiple QTL models, with a genome-dependent error rate of 0.05, which meant there was a 0.5 probability of missing a real QTL (Lander and Botstein 1989). For the purpose of comparing genomic regions with putative QTLs among the populations, RFLP segregation data from all four populations were pooled and a composite linkage map was constructed (Beavis and Grant 1991) (Fig. 1).

Plant height data were evaluated in the inbred parents, F_1 hybrids, and among the F_4 progeny from F_2 plants (which are referred to as $F_{2:4}$ lines) (Table 2). Field plot desgin for the B73 × MO17 and B73 × G35 populations was a randomized complete block consisting of two replications of field plots located in six central U.S. corn belt environments in 1987 and 1988. Each field plot included 50 plants grown in two rows that were 5.3 m long with 0.75 m between rows. Field plots for the J40 × V94 and K05 × W65 experiments were completely randomized in single replications located in six environments in North America in 1988. Each field plot included 25 plants grown in a single row that was 5.3 m long with 0.75 m between plots. The progeny of each line comprised an entry that was planted in a field plot and evaluated for plant height as the visual average height (centimeters to the top of the tassles) of the plants in the plot. Phenotypic values for each line were calculated as the entry mean across environments and replications. An analysis of variance for plant height due to lines (G), environments (E), and G × E interactions was used to assess the proportion of the phenotypic means that was attributable to genotypic sources (repeatability).

Results

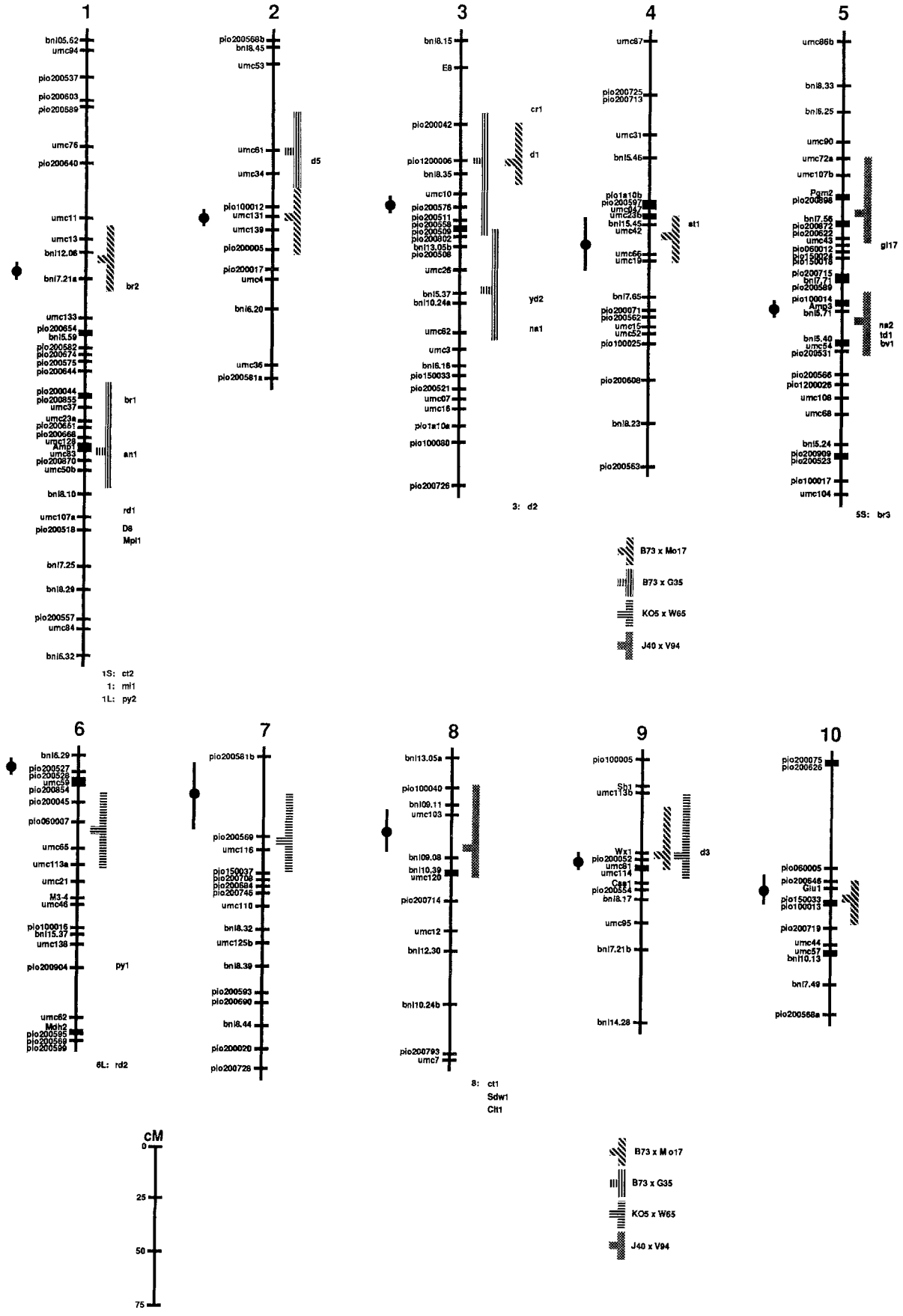
These populations were grown in a wide range of environments that included stressful drought conditions at several locations in 1988. Despite the large variability in environments, the repeatability of plant height values among families was high for all four populations (Table 2). A comparison of the average parental and F_1 hybrids indicated that plant height exhibited a heterotic effect of 30–40 cm. The average height among the $F_{2:4}$ lines was three to four times larger than would be expected if heterosis was due entirely to dominant expression at heterozygous loci.

Approximate locations of loci known to affect plant height and 90% QTL support intervals (SIs) are shown relative to our composite RFLP linkage map in Fig. 1. On average, the SIs encompass about 20 cM. Based upon RFLP and conventional marker linkage maps reported in the 1991 Maize Genetic Cooperation Newsletter (E. H. Coe) (personal communication), approximate locations of 18 qualitative genetic loci were determined relative to our map. There are nine additional qualitative loci that affect plant height, but these are localized, at best, to chromosome arms. Obviously, associations between these nine loci and our putative QTLs are not possible.

QTLs were identified in chromosome regions known to have qualitative genetic loci affecting plant height (Fig. 1). No QTL was consistently identified in all four populations, although QTLs identified on chromosomes 3 and 9 were present in two populations. The QTLs identified on chromosome 3 were consistent for populations with the same female parent, B73. However, there were eight QTLs in these two populations that they did not have in common. There were no QTLs identified in these populations in close proximity to *rd1*, *D8*, *Mpl1*, *d5*, or *py1* (Fig. 1).

Four of six QTLs identified in B73 × MO17 may be within 10 cM of qualitative genetic loci on chromosomes, 1, 3, 4 and 9. Ninety percent of SIs probably include the qualitative genetic loci *br2*, *d1*, *st1*, and *d3*. Two of the QTLs identified on chromosomes 2 and 10 in the B73 × MO17 population are not associated with previously mapped qualitative genetic loci, although *d5* is in close proximity to the QTL of chromosome 2. There are no known qualitative plant height loci on chromosome 10, although there is a narrow leaf mutant, *nl1*, and narrow leaf mutants are typically associated with reduced plant height. Further investigation of the association between the expression of plant height and segregation of RFLP loci on chromosome 10 showed that the association was very strong during 1988, a year with

Fig. 1. An RFLP map based on segregation data pooled from four F_2 populations showing QTL and 90% SI for plant height and approximate locations of mutations known to affect plant height


1
bnl05.62
umc94
pio200537
pio200603
pio200589
umc76
pio200640
umc11
umc13
bnl12.06
bnl7.21a
br2
umc133
pio200654
bnl5.59
pio200582
pio200674
pio200575
pio200644
pio200044
pio200855
umc37
umc23a
pio200651
pio200668
umc129
An1
umc83
pio200870
umc50b
br1
an1
bnl8.10
rd1
umc107a
pio200518
D8
Mpl1
bnl7.25
bnl8.29
pio200557
umc84
bnl6.32
1S: ct2
1: mi1
1L: py2
2
pio200566b
bnl8.45
umc53
umc61
umc34
pio100012
umc131
umc139
pio200005
pio200017
umc4
bnl6.20
umc36
pio200581a
d5
3
bnl8.15
E8
pio200042
pio1200006
bnl8.35
umc10
pio200576
pio200511
pio200559
pio200506
pio200802
bnl13.05b
pio200508
umc26
bnl5.37
bnl10.24a
umc82
umc3
bnl6.16
pio150033
pio200521
umc07
umc16
pio1a10a
pio100080
pio200726
cr1
d1
yd2
na1
3: d2
4
umc87
pio200725
pio200713
umc31
bnl5.46
pio1a10b
pio200597
umc94
umc230
bnl15.45
umc42
umc66
umc19
bnl7.65
pio200071
pio200562
umc15
umc52
pio100025
pio200608
bnl8.23
pio200563
st1
B73 x Mo17
B73 x G35
KO5 x W65
J40 x V94
5
umc86b
bnl8.33
bnl6.25
umc90
umc72a
umc107b
Pgm2
pio200696
bnl7.56
pio200672
pio200622
umc43
pio060012
pio150024
pio150018
pio200715
bnl7.71
pio200589
pio100014
Amp3
bnl5.71
bnl5.40
umc54
pio200531
pio200566
pio1200026
umc108
umc68
bnl5.24
pio200909
pio200523
pio100017
umc104
gl17
na2
td1
bv1
5S: br3
6
bnl6.29
pio200527
pio200528
umc59
pio200854
pio200045
pio060007
umc65
umc113a
umc21
M3-4
umc46
pio100015
bnl15.37
umc138
pio200904
umc62
Mdh2
pio200595
pio200569
pio200599
py1
6L: rd2
7
pio200581b
pio200569
umc116
pio150037
pio200788
pio200684
pio200745
umc110
bnl8.32
umc125b
bnl8.39
pio200593
pio200690
bnl8.44
pio200020
pio200728
8
bnl13.05a
pio100040
bnl09.11
umc103
bnl09.08
bnl10.39
umc120
pio200714
umc12
bnl12.30
bnl10.24b
pio200793
umc7
8: ct1
Sdw1
Clt1
9
pio100005
Sh1
umc113b
Wx1
pio200052
umc81
umc114
Cas1
pio200554
bnl8.17
umc95
bnl7.21b
bnl14.28
d3
B73 x Mo17
B73 x G35
KO5 x W65
J40 x V94
10
pio200075
pio200626
pio060005
pio200545
Glu1
pio150033
pio100013
pio200719
umc44
umc57
bnl10.13
bnl7.49
pio200568a
cM
0
25
50
75

severe heat and drought stress, but nonsignificant during 1987, a year with near-normal precipitation (data not shown). Whether this is truly a plant height response or is actually a drought tolerance response is unknown. We should emphasize that *Lte2*, responsible for drought tolerance, has been located toward the middle of chromosome 10. We have no evidence that supports or refutes the presence of *Lte2* in these materials.

All QTLs identified in B73 × G35 may be within 10 cM of qualitative genetic loci on chromosomes 1 and 3, and the 90% SIs for these QTLs probably include regions where *br1*, *an1*, *d5*, *cr1*, *d1*, *yd2*, and *na1* are mapped. The two QTLs identified in chromosome 5 in KO5 × W65 may be within 10 cM of *gl17*, *na2*, *td1*, and *bv1*. The remaining QTLs on chromosome 8 identified in this population may be associated with *ct1*, *Clt1*, or *Sdw2*, but these loci have yet to be mapped. Two of the QTLs identified in J40 × V94 may be within 10 cM of qualitative genetic loci on chromosomes 6 and 9. As with the QTL identified by Helentjaris and Shattuck-Eidens (personal communication) and one of the QTLs in B73 × MO17, a plant height QTL on chromosome 9 is in close proximity to the GA mutant *d3*. A third QTL in this population is located on chromosome 7, although there are no known qualitative plant height loci on chromosome 7.

Discussion

Plant height is generally considered to be a simply inherited trait with, at most, a few loci affecting the expression of the trait. Maize geneticists, however, have shown that there are at least 27 loci that can affect the quantitative expression of plant height (Sheridan 1988). It would seem that for the small populations we examined, the quantitative expression of plant height is controlled by a few polygenic loci (Table 3). However, 25–65% of the phenotypic variability of this highly heritable trait was not accounted for by significant QTLs. Populations size affects the number of QTLs that can be detected at some significance level (Soller et al. 1976; Lander and Botstein 1989). Thus, the amount of phenotypic variability accounted for by significant QTLs in these populations was not expected to be high. If we had used larger populations or accepted a higher error rate, more QTLs accounting for more phenotypic variability would be expected. Thus, the quantitative expression of plant height in these populations is probably controlled by a larger number of loci than we detected.

Plant height in maize is also considered to be a heterotic trait. In all four populations, there was a larger expression of heterosis from the $F_{2:4}$ lines than expected from the classic biometric concept (Falconer 1981). Ge-

Table 3. Genomic location, genetic effects, and cumulative percentage of phenotypic variability for plant height accounted for by QTLs in four maize populations

Population	Nearest RFLP locus	Chromosome	Distance[a] (cM)	Possible genetic loci	Estimated genetic effects[b]		Cumulative % Var
					Add.	Dom.	
B73 × MO17	bnl8.35	3	65	*d1*	−9.1	3.8	
	wx1	9	45	*d3*	6.1	5.0	
	umc131	2	85		−7.1	−4.1	
	pio150033	10	65		5.1	5.6	
	bnl12.06	1	105	*br2*	7.2	−5.3	
	umc42	4	100	*st1*	−6.9	−4.8	73
B73 × G35	umc83	1	185	*br1*, *an1*	−8.2	NS	
	umc61	2	55	*d5*	−7.8	−7.7	
	bn15.37	3	120	*yd2*, *na1*	−6.6	8.6	
	pio200006	3	65	*cr1*, *d1*	−6.4	NS	53
K05 × W65	bnl7.56	5	65	*gl17*	6.4	NS	
	pio1000014	5	145	*na2*, *td1*, *bv1*	5.8	NS	
	bnl10.39	8	50	*ct1*, *Sdw1*	5.8	NS	34
J40 × V94	pio200569	7	40		6.6	NS	
	umc81	9	45	*d3*	7.6	3.8	
	pio20095	6	40		7.1	NS	45

[a] Distance is measured from the terminal marker on the short arm of the chromosome

[b] All effects within a population were estimated simultaneously using MAPMAKER/QTL (Lander and Lincoln, unpublished). The sign of the estimated additive effects is associated with the allele from the male parent. For example, the estimated additive effect for the first QTL listed (−9.1) indicates that the allele from MO17 is associated with families that are, on the average, 9.1 cm shorter. The sign associated with estimated dominance effects indicates the effect of the allele from the male parent for the heterozygous condition. For example, the first QTL has an estimated dominance deviation of 3.8 cm, which indicates that the heterozygotes are 3.8 cm taller than expected, based upon the estimated additive effects (−9.1 cm) of the MO17 allele

netic explanations for heterosis include overdominance within loci, accumulation of dominant alleles at different loci, and epistasis (Hallauer and Miranda 1981). In the two populations where B73 was used as the female parent, there was significance associated with both overdominance and dominance estimates for most of the QTLs (Table 3). Recall, however, that plant height was evaluated from a sample of F_4s within each F_2-derived line. On the average, only one-fourth of the F_4 plants in an F_2 line that is heterozygous at a locus will be heterozygous. Thus, estimates of dominance effects are actually deviations from an additive model that could be due to either sampling or genetic expression.

Much of the theory on quantitative variation in population genetics is based upon the concept of multiple alleles at a locus (Kempthorne 1957). Our results indicate that very few QTLs were common across populations. One possible explanation of this result is that an array of alleles are possible at each QTL for plant height in maize germ plasm (Robertson 1984; Jensen 1989). Polymorphic alleles at QTLs in one population may be monomorphic in another. Also, it is possible that the effects on expression of plant height loci are genome dependent. Finally, it is possible that the QTLs identified are dependant upon the sample from the F_2 population.

Most of the 14 QTLs that we identified for these populations seem to be associated with qualitative genetic loci, which tends to support Robertson's hypothesis (1985) that major mutants studied by maize geneticists are actually null or near-null alleles at a QTL. Until the qualitative genetic loci are mapped relative to RFLPs and the information is pooled across mapping populations, the associations shown in Fig. 1 are subject to error. Indeed, our placement of qualitative genetic loci was done visually and may be prone to large error. Once the integration of RFLP and morphological markers has been accomplished, probability statements can then be made concerning the association between a QTL and the qualitative genetic loci. However, tests for allelism using molecular techniques will be necessary to confirm any putative associations.

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